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Rearing the Western Spruce Budworm

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This guide describes procedures for rearing large numbers of western spruce budworm, *Choristoneura occidentalis* Freeman, in the laboratory. The information is based on experience with a colony of insects started from larvae collected in Montana and Idaho in 1964 and maintained in Berkeley, California.

Until 1967, insects in the second larval stage of this laboratory colony diapaused. This period of arrested development could be ended only after months of refrigeration; as a result, only two generations could be raised per year. From 1964 to 1967, five generations were raised providing about 1,000 larvae of all stages each week for research purposes. In 1967, a nondiapausing colony was started by modifying the rearing conditions. Larvae developed continuously so that 7.5 generations could be produced each year. The nondiapausing colony, now in its eighty-first generation, has provided at least 5,000 larvae of each instar per week. Instructions for rearing the western spruce budworm both with and without diapause are provided.

Diapause rearing of other *Choristoneura—C. fumiferana* (Clemens), the eastern spruce budworm; *C. pinus pinus* Freeman, the jack-pine budworm; and *C. viridis* Freeman, the Modoc budworm—can be done as described for the western spruce budworm. Nondiapausing colonies can also be established, but the diapause requirement is less easily eliminated for these species. Fewer larvae develop without diapause in the initial generations so that five to six generations must pass before a nondiapause colony is firmly established.

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Physical Parameters

The temperature in the rearing laboratory must be maintained between 23 °C and 26 °C at all times. A 24-hour photoperiod (8 hours darkness, 16 hours light) permits normal development. During the light cycle, light intensity in the open areas of the laboratory can vary from 100 to 120 foot-candles. During the dark cycle, light intensity should not exceed 10 foot-candles. Containers of larvae, stacked on trays, may be kept on shelves where light intensity during the light cycle may be as low as 20 foot-candles without adverse effects.

Relative humidity can vary from 30 to 50 percent. Under extremely humid conditions (70 percent relative humidity and above) moisture condenses inside the rearing containers. Fungi and bacteria begin to grow on the artificial diet and small larvae are trapped in water droplets where they eventually drown.

Sanitary Procedures

Saprophytic fungi such as *Aspergillus, Rhizopus,* and *Penicillium,* and bacteria such as *Escherichia coli* thrive on artificial diet when sanitation is disregarded. Although these micro-organisms are not pathogenic, they are detrimental to the development and survival of the insects. Antimicrobial agents in the artificial diet help suppress fungi and bacteria, but are no substitute for good sanitation. Rearing containers in which fungi or bacteria have begun to grow should be discarded, regardless of age or condition of the insects.

Sterilization is most important at the egg stage and need not be done at other life stages if other sanitary precautions are followed. The eggs must be sterilized before they are placed in rearing containers. Immediately after harvest, stir the eggs gently for 15 minutes in 200 milliliters of 10 percent formalin plus one drop of a wetting agent such as Tween 20.[2] Follow the wash with two rinses (10 minutes each) in 200 milliliters of distilled water. Agitate at low speed during washing and rinsing with a

[2] Trade names and commercial enterprises or products are mentioned for information only. No endorsement by the U.S. Department of Agriculture is implied.

mechanical stirrer. Then remove the egg masses from the distilled water, gently separate them with forceps, spread them out on filter paper, and allow to air dry for 1 to 3 hours. Never leave water droplets on egg masses; excessive moisture inhibits hatching, drowns small larvae, and fosters the growth of microbes.

Sterilize forceps and brushes used to transfer larvae or pupae by autoclaving or chemical treatment. Brushes can be sterilized with propylene oxide under a fume hood. Forceps and small spatulas used to cut artificial diet can be washed with soap and water, dried in an oven, then autoclaved. When not in use, store forceps, brushes, and small spatulas in closed containers such as enamel pans with lids.

During use, wipe forceps often with a surface sterilant such as ethyl alcohol or hospital disinfectant such as Roccal[2] or Amphyl.[2] Never place or empty rearing containers directly on a table or counter surface. Rather, lay a sheet of blank newsprint on work surfaces.

Replace the newsprint frequently each day to keep organic debris from accumulating in work areas. Empty individual containers onto white tissue such as Kimwipes[2] or Kleenex[2] and discard each tissue after use.

Scrub work areas after each use with a hospital disinfectant or a dilute solution of sodium hypochlorite (Clorox[2]). Do not use, then reuse, cloth towels or sponges for this purpose because they tend to spread contamination. Paper towels are preferable for routine cleaning.

Keep the equipment used to make artificial diet scrupulously clean. Wash containers thoroughly and sterilize them by dry heat before each use. Wipe the casings of electrical equipment with disinfectant to remove diet that may have splashed there. If the diet must be handled, wear sterile disposable gloves.

Preparation of Artificial Diet

Artificial diet can easily be made in quantities appropriate for any number of insects in a western spruce budworm colony. For example, three 15,000-gram batches of diet made over a two-week period is enough to set up rearing containers for about 50,000 larvae and feed larvae removed from the culture for experimental uses. Once made, diet can be stored for as long as 2 weeks at 4°C.

Equipment

For making 15,000-gram batches, the following equipment is needed: a commercial mixer with a 5-gallon (18.93-liter) mixing bowl, a flat beater attachment, and timed, variable speed controls; two 1.5- to 2-liter stainless steel pots; a commercial three-speed blender with a 1-gallon (3.79-liter) container; four metal trays, each at least 2.5 centimeters deep, 48 centimeters long, and 35 centimeters wide; and miscellaneous weighing containers.

For less than 3,750 grams of diet, all mixing can be done in a 1-gallon blender and only one tray is needed to hold the diet as it solidifies.

Ingredients (per 1,500 grams of diet):

Group A
Distilled water—930 ml
Agar[3]—40.5 g
Vitamin-free casein[4]—58.5 g
Alphacel[4]—9.0 g
Salt mixture W[4]—15.0 g
Wheat embryo[5] (grind before use)—49.5 g

Group B
Sucrose—43.5g
Distilled water—250 ml
4 M potassium hydroxide—10.0 ml
55 percent linolenic acid[4]—3.0 ml
Vitamin diet fortification mixture[4]—18.0 g
Ascorbic acid—6.0 g
Methyl parahydroxybenzoate[4] —2.55 g
Potassium sorbate (sorbic acid potassium salt, 99 percent)[4] —1.8 g
Aureomycin (14.1 percent chlorotetracycline)[6]—1.4 g
Formalin (37 percent)—0.9 ml

[3] Difco Bacto Agar, Difco Laboratories, Detroit, Michigan.

[4] ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, Ohio.

[5] Biogram Foods Ltd., 101 Shornliffe Road, Toronto 18, Ontario. Do not substitute wheat germ for wheat embryo.

[6] Harvest Industries Inc., Cutting Division, Sacramento, California.

Procedure for Mixing Diet

1. Autoclave all ingredients in group A together at 15 pounds per square inch for 50 minutes at the liquid setting. For a large batch of diet, such as 15,000 grams, mix the ingredients in group A together, divide the mixture into equal portions, then autoclave them in two 1.5- to 2-liter stainless steel pots. This procedure assures that the agar will melt completely. Grind the wheat embryo in a blender before use so it won't sink to the bottom of the autoclaving pans. Ingredients 2 through 6 can be weighed, mixed together, and stored for future use.

2. While ingredients in group A are being autoclaved, measure those in group B and mix in the blender.

3. Remove mixture A from the autoclave, pour it in the mixer bowl (fig. 1), and stir with the flat beater attachment.

4. When the temperature cools to 60°C, add the ingredients from group B.

5. Stir this mixture at low speed until it cools to from 43°C to 45°C, then pour it to a depth of about 2 centimeters into trays lined with heavy duty aluminum foil. Do not pour the diet at temperatures above 45°C or the wheat embryo will settle during cooling. Below 43°C, the agar forms gelatinous lumps.

The diet is ready to cut and use about 1 hour after it has been poured. If the diet is to be used later, wrap it in aluminum foil (fig. 2) and store in the refrigerator. Use new pieces of foil and discard the foil lining the trays.

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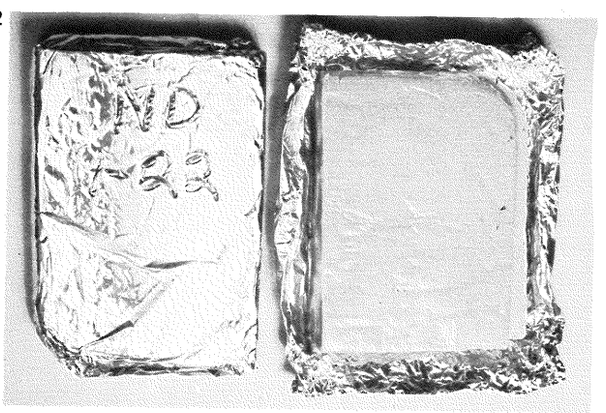

Figure 1.—Preparation of artificial diet.

Figure 2.—Artificial diet cut and wrapped for storage.

Establishing a Laboratory Colony

Western spruce budworm larvae, particularly instars 4 through 6, that have been collected in the field will readily feed on artificial diet. Carefully remove the larvae from the foliage with forceps. Place no more than 10 larvae of about the same size in each 100- by 15-millimeter or 100- by 20-millimeter sterile plastic petri dish with artificial diet. Select only healthy insects. Transfer of some plant material is unavoidable, but should be minimized to prevent contamination of the diet.

Some larvae transferred from foliage to artificial diet will die when parasites emerge. Others will be injured fatally by the mechanical process of the transfer. Of those which pupate, some will die, probably as a result of handling. At least 300 must survive to the pupal stage to establish a small colony.

White, untanned, pupae are extremely fragile and easily injured. Therefore, pupae should be removed from petri dishes as soon as possible, but only after their cuticles have tanned and hardened. As pupae are collected, determine their sex by counting the number of abdominal segments visible on the abdomen behind the wing pads.

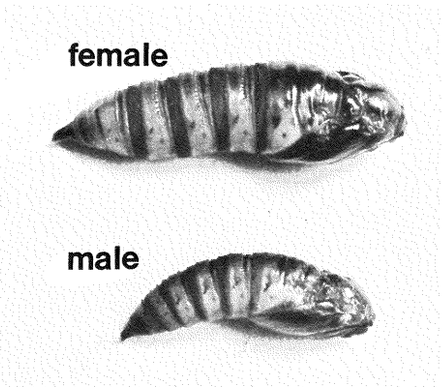

Figure 3.—Male and female (above) pupae of the western spruce budworm.

Viewed from the side or bottom, five segments are visible in males and four in females (fig. 3).

Set up single pair or multiple pair matings after pupae have been collected and sexed. For the first several generations, single pair matings are preferable to mass matings unless extremely large numbers of pupae are available. Male and female pupae placed in each mating container must be of approximately the same age so that adults will emerge at about the same time; egg production is higher when adults mate within one day after emerging. Eight-ounce paper cups with plastic lids (for

example, Dixie[2] cold drink cups) are ideal single-pair mating containers. Small unwaxed brown paper bags (No. 6) can be used for mating 10 to 15 pairs. Sprigs of fresh Douglas-fir foliage or strips of waxed paper placed in each cup or bag provide a substrate on which the insects will lay eggs. After the containers are closed, place them in a darkened cabinet. Harvest the eggs from each container 10 days to 2 weeks later.

If sufficient adults emerge and mate to produce from 30 to 50 large egg masses, a western spruce budworm colony has successfully passed through its parent or *P,* generation. A rearing method for the F_1, or first filial, generation must be chosen. These insects may be reared with or without diapause.

Figure 4.—Larvae remain in a single cup through all stages of development. Container at far left contains an egg mass which has not yet hatched. Next, first instars have hatched and begun to feed. The next cup contains third and fourth instars. The container at far right is filled with sixth instar larvae.

During nondiapause development, western spruce budworm not used to propagate the colony spend their larval life in the same 7-ounce (200-ml) clear plastic disposable specimen container in which they hatched from egg masses (fig. 4).

1. Fill each container ⅓ to ½ full of cubes of artificial diet 1- to 1.5-centimeters in each dimension.

2. After eggs have been disinfected and dried, place a single egg mass of 50 to 100 eggs near the bottom of each cup. As an alternative, fill each cup 1/6 to 1/4 full and add fresh diet when larvae reach the third or fourth instars.

3. Firmly press an unwaxed paper lid into the top against the inner rim to close each container. Unwaxed lids allow some moisture to evaporate from the diet and help prevent the condensation of moisture on the sides of the rearing cup. Lids made to fit the containers have side tabs; these should be folded back or removed so the edge of each lid is flush with the cup.

4. Cover each cup with a piece of aluminum foil so the sides of the cup are covered to the top of the diet (fig. 5). Punch holes in the foil over the top of the cup to aid ventilation.

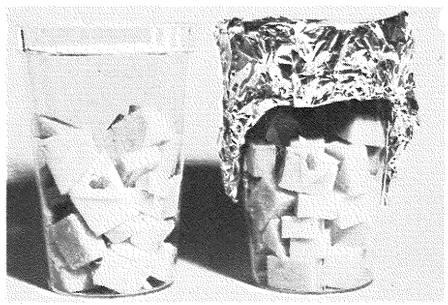

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Figure 5.—Rearing containers are covered with aluminum foil to use light to attract larvae to the diet.

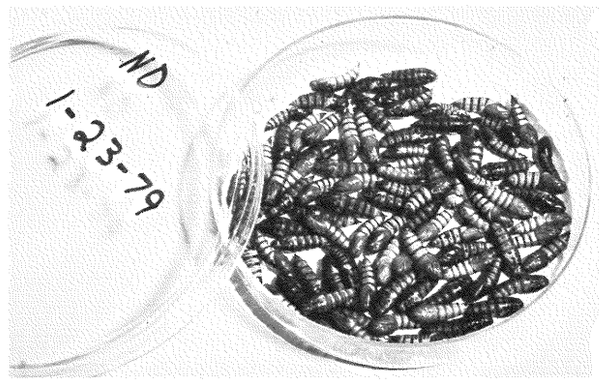

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Figure 6.—Pupae are collected from propagation dishes, their sexes determined, and mass mating containers prepared.

The exclusion of light from the upper area of the rearing cup seems to be the key to rearing the western spruce budworm without diapause. Newly hatched larvae will be attracted to the top level of diet where light enters the cup. There they begin to feed. In the first few generations of nondiapause rearing, most first instars will migrate to the top of a rearing cup, spin hibernacula along the cup-lid interface, and diapause if a light-excluding covering such as foil is not placed over the top of the cup.

As a result of staggered matings in the P or F_1 generations, a nondiapause colony will eventually consist of groups of insects in all developmental stages. For propagating the colony each week, set aside a subsample of fifth and sixth instars. To maintain a large colony, transfer 600 to 900 of these larvae from rearing containers to 100- by 20-millimeter or 100- by 15-millimeter sterile plastic petri dishes containing pieces of artificial diet. Place about 10 larvae in each dish. Once a week, harvest and sex the pupae (fig. 6), and set up three mass mating containers.

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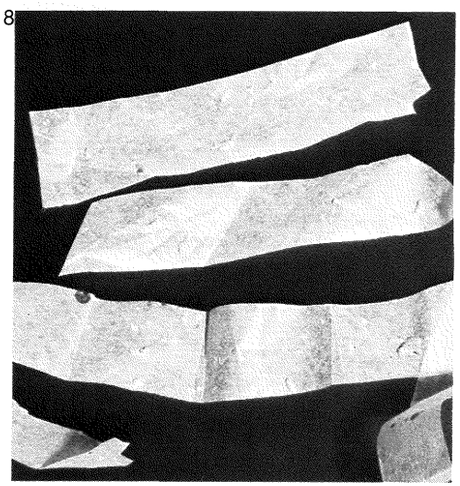


Figure 7.—A mass mating container with waxed paper strips and pupae.

Figure 8.—Egg masses are cut off the waxed paper strips before they are disinfected.

Place 50 to 80 pairs of male and female pupae in each mass-mating container—a No. 46 Kraft paper bag with randomly arranged waxed paper strips (fig. 7). These strips, about 2.5 centimeters wide and 36 centimeters long, provide an excellent surface for egg laying. Fold each bag at the top and staple shut. Single pair matings, described previously, may be necessary for some studies.

In their natural environment, western spruce budworm fly and mate at dusk. In the laboratory, the provision of darkness during mating seems to satisfy the insects' behavioral preference. Sufficient darkness within the mass mating bags is provided by the heavy brown paper. When 8-ounce cups with plastic lids are used for single pair matings, however, they must be placed in a darkened area. Put all mating containers in a storage cabinet with the doors closed to prevent light from reaching the mating containers.

After 10 days to 2 weeks, remove the mating containers from the cabinet. Anesthetize adults with CO_2 pumped into each bag through a small hole. Open the bag and remove the waxed paper strips. Cut the individual egg masses off the waxed paper (fig. 8) and wash the eggs. Disinfected egg masses can be refrigerated at 10°C for up to a week without detrimental effects if there is a surplus from one week to the next.

Larval development without diapause normally requires about 30 days—9 days in the first instar, 5 days in the second instar, 3 days in the third instar, 4 days in the fourth instar, 3 days in the fifth instar, and 6 days in the sixth instar. Insects not used by the end of the larval period should be discarded unless research is planned with pupae or adults.

The pupal stage lasts about 7 days and the adult stage about 5 days. From egg to egg, generation time is about 42 days. To this is added the average 7-day period required for egg hatch, making the total interval between generations about 7 weeks.

Survival of western spruce budworm reared without diapause is usually about 25 percent. About 16 percent of the eggs do not hatch, 48 percent of the larvae do not live to become pupae, and 11 percent of the pupae do not develop into adults. Even with this seemingly low survival rate, the number of insects in the population increase 10-fold every 7 weeks. In the early generations, fewer eggs can be placed in each rearing container to increase larval survival. When a colony has reached the desired size, the 25 percent survival provides more than enough insects.

The activities necessary for maintaining a nondiapause colony can be arranged on a Monday through Thursday schedule:

Monday

1. Harvest eggs from mating bags set up 12 days earlier.
2. Wash the eggs and store at 10°C.
3. Pick pupae from propagation colony and store at 10°C.

Tuesday

1. Set up eggs in rearing containers.

Wednesday

1. Set up propagation colony in petri dishes.
2. Set up mass matings with the pupae picked on Monday.

Thursday

1. Dispose of excess rearing containers holding older larvae and pupae.

Rearing With Diapause

1. Place each disinfected egg mass in the bottom of a 100- by 20-millimeter sterile plastic petri dish.

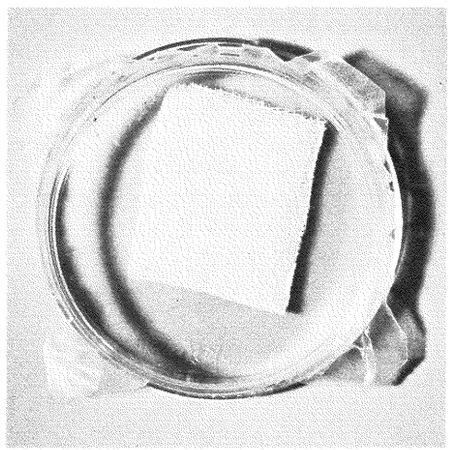
2. Place a cover of Parafilm[2] (onto which a 30- by 30-mm square of sterile surgical gauze has been pressed) over the bottom of the petri dish and then place the top of the dish over the Parafilm (fig. 9).

3. Remove the lug vents from the top of each petri dish so that small larvae cannot escape.

4. Place the petri dish inside a small black paper bag.

5. Cut a window out of the bag just behind the gauze (fig. 10). Place a light source above the window so that light enters the dish through the top. When first instars hatch, they are attracted into the gauze by the light. There they spin hibernacula, molt to the second instar, and diapause.

6. Hold each petri dish at room temperature for 3 weeks, 4-5°C for 1 week, then 0°C for at least 16 weeks. A period of cold storage of about 200 days results in highest survival.



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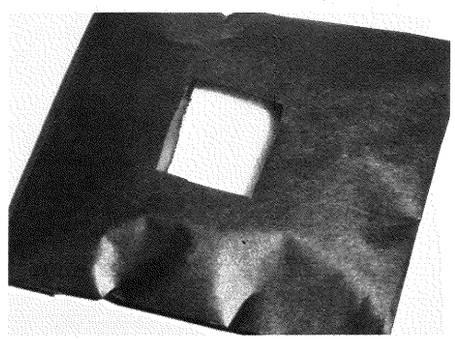

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Figure 9.—Petri dishes arranged for diapause rearing. Parafilm with gauze is placed over the bottom of the petri dish. The top of this dish is then placed over the parafilm. First instars will diapause in the gauze.

Figure 10.—Rearing containers are placed in a black bag with a hole cut in the top to let in light. When they emerge, first instars are attracted by light into the gauze, where they spin their hibernacula.

Several steps are necessary to promote insect development following cold storage:

1. Remove the black bag.

2. Place a small cube of diet in the bottom of the dish. The diet should not touch the gauze; if it becomes saturated with moisture from the diet, fungi may begin to grow.

3. Close the dish, seal it with cellophane tape, and put it back in the black bag. Make sure that the position of the window in the bag is now reversed. Light should enter the bottom of the petri dish from behind the diet.

After about 3 weeks, larvae will emerge from the gauze and migrate to the diet. They can then be transferred to rearing containers (petri dishes or specimen containers) with sterile No. 3 camel's hair brushes. As an alternative, each square of gauze can be pulled off the Parafilm with forceps and put in a specimen container. After the larvae emerge, remove the gauze from the rearing container. Development through the life stages continues for about the next 23 days.

The diapause technique requires staggered single or multiple pair matings if all larvae are chilled for 200 days and a supply of insects in all stages is needed. Mass-matings are not usually possible unless the diapause colony is very large. However, diapausing larvae may be removed from refrigeration anywhere from 100 to 250 days.

Since survival of western spruce budworm reared with diapause is usually no better than for those reared without, and the insects must be handled more often, there is no obvious advantage to using the diapause technique.

Quality of Laboratory-Reared Insects

Mass-reared western spruce budworm, particularly those produced after long-term culture, constitute an isolated population which has evolved in an artificial environment. Selection pressures on laboratory-reared insects are vastly different from those acting on a wild population in its natural environment. Some divergence from the characteristics of field-collected insects must be expected.

For example, a western spruce budworm colony reared without diapause for about 20 generations cannot revert to development with diapause. For the first few generations, however, alternate diapause and nondiapause rearing of successive generations is possible. Thus, a rearing method need not be selected immediately after establishment of the laboratory colony.

The insects which form the basis of a continuing colony appear morphologically normal when reared by these guidelines. Malformed pupae and adults are rare. Adults fly and mate normally; their pheromone production and reception are assumed to be comparable to that of insects in the field. Larvae in nondiapause culture tend to grow larger in the last stage than wild larvae. This may be because of the abundant food supply in the laboratory. The capacity of larvae to eat the same type of host foliage as their ancestors, however, is not lost. Even after 81 laboratory generations, nondiapausing larvae readily consume Douglas-fir foliage.

The responses and characteristics of laboratory insects should be compared with those of insects collected from the field as frequently as possible during a long-term rearing program. A genetic comparison can be made by starch gel electrophoresis. If necessary, new genetic stock from wild populations can be incorporated into the laboratory culture.

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